

# Mass Spectrometry Techniques in Emerging Pathogens Studies: COVID-19 Perspectives

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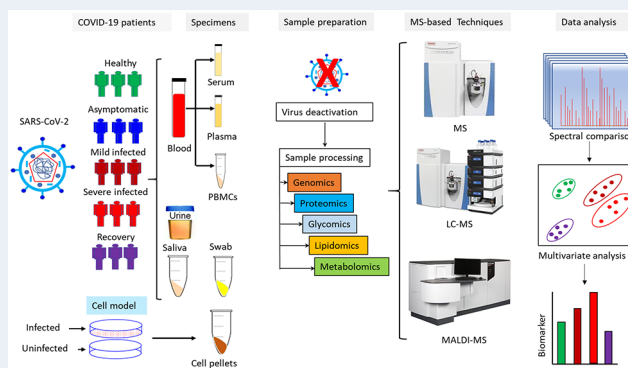
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**ABSTRACT:** As corona virus disease 2019 (COVID-19) is a rapidly growing public health crisis across the world, our knowledge of meaningful diagnostic tests and treatment for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still evolving. This novel coronavirus disease COVID-19 can be diagnosed using RT-PCR, but inadequate access to reagents, equipment, and a nonspecific target has slowed disease detection and management. Precision medicine, individualized patient care, requires suitable diagnostics approaches to tackle the challenging aspects of viral outbreaks where many tests are needed in a rapid and deployable approach. Mass spectrometry (MS)-based technologies such as proteomics, glycomics, lipidomics, and metabolomics have been applied in disease outbreaks for identification of infectious disease agents such as virus and bacteria and the molecular phenomena associated with pathogenesis. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) is widely used in clinical diagnostics in the United States and Europe for bacterial pathogen identification. Paper spray ionization mass spectrometry (PSI-MS), a rapid ambient MS technique, has recently open a new opportunity for future clinical investigation to diagnose pathogens. Ultra-high-pressure liquid chromatography coupled high-resolution mass spectrometry (UHPLC-HRMS)-based metabolomics and lipidomics have been employed in large-scale biomedical research to discriminate infectious pathogens and uncover biomarkers associated with pathogenesis. PCR-MS has emerged as a new technology with the capability to directly identify known pathogens from the clinical specimens and the potential to identify genetic evidence of undiscovered pathogens. Moreover, miniaturized MS offers possible applications with relatively fast, highly sensitive, and potentially portable ways to analyze for viral compounds. However, beneficial aspects of these rapidly growing MS technologies in pandemics like COVID-19 outbreaks has been limited. Hence, this perspective gives a brief of the existing knowledge, current challenges, and opportunities for MS-based techniques as a promising avenue in studying emerging pathogen outbreaks such as COVID-19.

**KEYWORDS:** mass spectrometry, emerging pathogen, COVID-19, proteomics, glycomics, metabolomics and lipidomics



## INTRODUCTION

The etiology of a novel type of coronavirus was identified on January 7, 2020, in Wuhan City in the Hubei Province of China.<sup>1</sup> This novel coronavirus (CoV) was later named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease it causes was called coronavirus disease 2019 (COVID-19).<sup>2</sup> Originally, the COVID-19 epidemic was concentrated in China, but it rapidly spread around the world with approximately 15,547,081 known cases and 633,142 deaths as of July 23, 2020.<sup>3</sup> COVID-19 is now one of the most devastating pandemics that has affected public health on all six populated continents. There are four known genera of CoV, alpha, beta, gamma, and delta that cause a multitude of symptoms including severe respiratory issues, nervous system alterations, immune dysfunction, as well as kidney, cardiovascular, lung, and gastrointestinal malfunction.<sup>4–10</sup> According to

recent studies, SARS-CoV-2 binds to the angiotensin converting enzyme II (ACE2) receptors of the cell of nasal epithelia, lungs, or other organs and triggers an immune response which is believed to ultimately lead to symptoms.<sup>11,12</sup> However, in many cases, individuals have no symptoms that lead to COVID-19 but can still carry and transmit the virus for up to 14 days leading to profound exposure concerns.<sup>13,14</sup> Several research studies have suggested that COVID-19 is a silent spreader and can be divided into asymptomatic where

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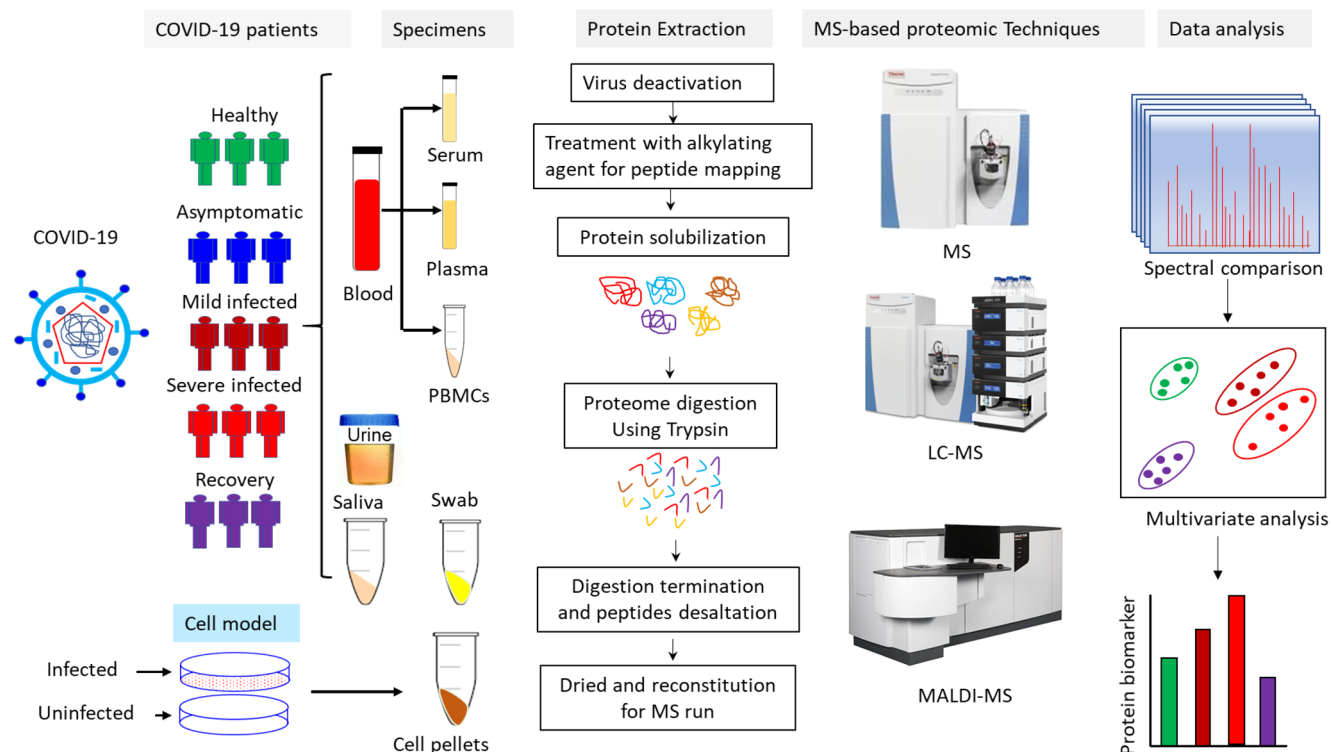
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**Table 1. Summary of MS-Based Proteome, Lipidome, and Metabolome analyses in COVID-19 Patients**

proteomics in COVID-19 study	technique (sample)	sample preparation	sources
Identified protein–protein interactions between SARS-CoV-2 and human proteins	Affinity purification LC–MS (Vero E6 and HEK 293T cells)	Samples were resuspended in 4% formic acid and 2% acetonitrile solution and separated by a reversed-phase gradient over a Nanoflow C18 column. Q-Exactive Plus mass spectrometer was used for data acquisition.	38
Identified peptide sequence from SARS-CoV-2 nucleoprotein	Nano-HPLC/Nano-ESI-Orbitrap-MS/MS (gargle solution)	The protocol consists of an acetone precipitation and tryptic digestion of proteins contained within the gargle solution.	44
Downregulated: APOA1, APOA2, APOH, APOI, APOD, and APOM	TMT MS (serum)	Ethanol used for virus inactivation, serum was lysed using 50 $\mu$ L of lysis buffer (8 M urea in 100 mM 680 triethylammonium bicarbonate, TEAB), reduced in 10 mM tris(2-carboxyethyl)phosphine (TCEP), alkylated by 40 mM iodoacetamide (IAA) in darkness, dilution done with 100 mM TEAB, and digestion with double-step trypsinization.	39
Downregulated: NPC2, APOA1, and CUBN Upregulated: C3, CREB3L3, HYOU1, and SERPIND1	LC–MS/MS (urine)	5 mM dithiothreitol (DTT) used for virus inactivation. Urine samples were alkylated with 10 mM iodoacetamide in darkness. Proteins were digested with trypsin, and then the digestion reaction was terminated by 1% formic acid (FA) and the digested peptides were desalted and dried before LC–MS/MS analysis.	40
Detected tryptic peptides of SARS-CoV-2 proteins (NCAP, VME1)	Orbitrap MS (Vero E6 cell)	Infected cells were boiled at 95 °C for 20 min to inactivate the virus. Cells were lysed with cell lysis buffer, and proteins were reduced and alkylated with DTT and IAA reagent, respectively, and precipitated using chloroform/methanol.	41
Upregulated: A1BG, ACTB, C1R, C1S, C8A, CD14, CFB, CFH, CFI, CRP, FGA, FGB, FGG, HP, ITIH3, ITIH4, LBP, LGALS3BP, LRG1, SAA1, SAA2, and SERPINA10 Downregulated: ALB, APOA1, APOC1, TF, and GSN	LC–MS (plasma/serum)	8 M urea, 100 mM ammonium bicarbonate (ABC), and 4.5 mM dithiothreitol (DTT) were used for plasma/serum sample denaturation/reduction. IAA was used for alkylation, and digestion was carried out with trypsin.	42
Identified nsp9 and nsp10 protein of SARS-CoV-2 interact with host NKRF	LC–MS (patients PBMC)	PBMC cells were lysed by urea buffer to denature the proteins and sonicated twice on ice to break the protein–protein or DNA–protein interactions. TCEP (5 mM) and iodoacetamide (10 mM) were used for reduction and alkylation, respectively.	43
Spectral comparison and multivariate analysis identified distinct cluster between SARS-CoV-2 detectable versus undetectable nasal samples.	MALDI-TOF (nasopharyngeal swabs samples)	1 $\mu$ L of sample + 1 $\mu$ L of HCCA matrix (a solution containing $\alpha$ -cyano-4-hydroxycinnamic acid diluted into 500 $\mu$ L of acetonitrile, 250 $\mu$ L of 10% trifluoroacetic acid, and 250 $\mu$ L of HPLC grade water) for protein extraction.	46
Upregulated: Nucleotide metabolism pool	LC–MS (Caco-2 cell)		45
glycomics in COVID-19 study	technique (sample)	sample preparation	sources
Identified N-, O-, N-and O-Glycosylation and Fucosylation on hACE2	Ion-Trap MS (HEK 293 cell)	Purified hACE2 was expressed in HEK293 cells in 50 mM ammonium bicarbonate solution. Sample reduction, alkylation, and digestion were done by 25 mM DTT, 90 mM IAA, and trypsin, respectively.	49
Identified glycan mimic polypeptide epitope that modulate SARS-CoV-2 spike protein and host ACE2 receptor interactions	LC–MS (HEK 293F cell)		50
lipidomics in COVID-19 study	technique (sample)		sources
Upregulated: Diglycerides (DGs), free fatty acids (FAAs), and triglycerides (TGs) Downregulated: Phosphatidylcholines (PCs)	LC–ESI–MS/MS (Plasma)	50 $\mu$ L of plasma/serum homogenized with 1 mL solvent mixture (include methanol, MTBE and internal standard mixture) for lipid extraction.	95
Upregulated: Phosphatidylcholine (PC) and 21-hydroxypregnenolone, the essential intermediate for synthesizing corticosterone Downregulated: Glycerophospholipid and sphingolipids Upregulated: Hyperlipidemia	UPLC–MS/MS (Sera)	Serum samples were treated with ethanol for viral deactivation. Ice-cold 100% methanol was used for metabolite extraction.	39, 129
metabolomics in COVID-19 study	technique (sample)		sources
Discriminated metabolites: TCA cycle, carbamoyl phosphate of urea cycle, and guanosine monophosphate (GMP) of nucleotide biosynthesis pathways.	LC–ESI–MS/MS (Plasma)	3 volumes of ice-cold methanol were added to 1 volume of plasma/serum for protein precipitation and metabolite extraction.	95
Upregulated: kynurenine pathway with elevated kynurenate, kynurenine, 8-methoxymethoxykynurenate in COVID-19 patients. Downregulated: choline and its derivative phosphocholine as well as global suppression of amino acids and derivatives such as glutamate, arginine, N-(L-arginino)succinate, citrulline, ornithine, glutamine, 2-oxoglutarate, N-acetyl-L-glutamate, urea, fumarate, arginate, asymmetric dimethylarginine, symmetric dimethylarginine, homoarginine, and N-acetylarginine	UPLC–MS/MS (Sera)	Serum samples were treated with ethanol for viral deactivation. Ice-cold 100% methanol was used for metabolite solubilization and extraction.	39
Upregulated: kynurenine, glucose, and free fatty acids Downregulated: Nitrogen metabolism including creatine, creatinine, polyamine, and circulating amino acids	UHPLC–MS (Sera)	Sera were extracted via a modified Folch method (chloroform/methanol/water 8:4:3). Twenty $\mu$ L of serum was diluted in 130 $\mu$ L of LC–MS-grade water and then 600 $\mu$ L of ice-cold chloroform/methanol (2:1) mixture were used for metabolite extraction.	130
Identified NAD dysregulation in COVID-19 patient's	LC–MS/MS (A549 cell)		98

people carry the active virus but never develop any symptoms, presymptomatic, where people have been infected and are incubating the virus but do not show symptoms, and mildly

symptomatic where people feel slightly unwell from a COVID-19 infection but continue to come in close contact with others.<sup>14,15</sup> According to a recent global health report, 1 in 5



**Figure 1.** MS-based proteomics to study COVID-19. Workflow developed based on recent proteomic analysis on COVID-19 pandemic. SARS-CoV-2 virus with different symptomatic patient conditions or infected cell model are shown. Typically, serum, plasma, and PBMCs from blood as well as urine, saliva, and nasal swabs have been utilized for targeted or untargeted proteomic analysis in COVID-19 patients. MS spectral comparison followed by multivariate statistical analysis can be applied for biomarker analysis with different levels of COVID-19 severities. For details about different COVID-19 proteomic sample preparation, data collection, and analysis, see Table 1.

people worldwide have an underlying health condition that could increase their risk of severe COVID-19.<sup>16</sup> Several risk factors have been identified as associated with COVID-19 and its disease severity, including older age, asthma, underlying respiratory disorder, hypertension, cardiac disease, renal disease, diabetes, obesity, cancer, and smoking.<sup>17–20</sup>

Currently, there is no meaningful vaccine or treatment for this novel coronavirus disease other than repurposed therapeutics that exhibit some beneficial outcomes for severe illness.<sup>21</sup> For example, the use of dexamethasone resulted in lower mortality among those who were receiving either invasive mechanical ventilation or oxygen alone but not among those receiving no respiratory support.<sup>22</sup> COVID-19 can be diagnosed using RT-PCR; however, due to inadequate access to reagents and equipment, disease detection and management have been slowed.<sup>23,24</sup> Precision medicine, individualized patient care, requires suitable diagnostic approaches to tackle the challenging aspects of virus outbreaks where many tests are needed in a rapid and deployable approach.

According to the World Health Organization (WHO), outbreaks are inevitable and often unpredictable events.<sup>25</sup> The immediate priority for COVID-19 diagnostic research is the development of oligonucleotide and serological tests as well as detection with point-of-care devices.<sup>26</sup> The long-term priority for studying outbreaks such as COVID-19 is to integrate these existing techniques into multiomics approaches to enable clinicians/researchers to better track healthy, sick, and recovered patients.

Mass spectrometry (MS)-based integrated omics includes proteomics, glycomics, metabolomics, and lipidomics studies

which provide a comprehensive snapshot of pathogen-induced changes to the host following infection, invasion, persistence, and pathogenesis and can prime the identification of novel therapeutic targets for preventing or lessening disease severity.<sup>27,28</sup> Pathogen infection can be primed through the manipulation of host metabolism.<sup>29</sup> Adaptation into the host metabolic landscape is a prerequisite for viral pathogen to replicate, thrive, and out-compete neighboring cells that they invade. Therefore, understanding the role of metabolism through viral pathogenesis is valuable for next generation therapy development. Here, we will provide current integrated knowledge of MS-based genomics, proteomics, glycomics, lipidomics, and metabolomics technologies in emerging pathogen studies, specifically focused on the opportunities and challenges related to the COVID-19 pandemic.

## ■ MASS SPECTROMETRY-BASED PROTEOMICS IN COVID-19

MS-based proteomic approaches such as liquid chromatography (LC)–MS and matrix-assisted laser desorption/ionization (MALDI)–MS have been successfully utilized in clinical settings as diagnostic tools for investigating viruses and their pathogenesis.<sup>30,31</sup> Proteomics approaches have been applied successfully in studying pandemics and epidemics including dengue, chikungunya, Zika, Ebola, swine flu, H1N1 and other influenza, MERS-CoV, SARS, and SARS-CoV-2.<sup>32–37</sup> Endogenous proteins serve as receptors for the virus to enter host cells, and thus, elucidation of the proteome has made attractive contributions to antiviral therapies.<sup>12</sup>

As a pioneering study in COVID-19, using affinity purification mass spectrometry, Gordon et al. identified 332



high-confidence protein–protein interactions between SARS-CoV-2 and human proteins and suggested druggable targets to treat COVID-19.<sup>38</sup> Using stable isotope labeled Tandem Mass Tag (TMT) MS-based proteomics, Shen et al. identified dysregulation of multiple apolipoproteins including APOA1, APOA2, APOH, APOB, APOE, and APOC in patient's sera.<sup>39</sup> Using LC–MS/MS-based proteomics of urine specimens from COVID-19 patients, Li et al. found mild and severe COVID-19 patients with comorbidities were clearly differentiated from healthy patients.<sup>40</sup> This study identified that intracellular cholesterol transporter 2 (NPC2), APOA1, and cubilin (CUBN) were downregulated in severe COVID-19 patients, but cyclic AMP-responsive element-binding protein 3, like protein 3 (CREB3L3), hypoxia up-regulated protein 1 (HYOU1), heparin cofactor 2 (SERPIND1), and complement 3 (C3), were upregulated in mild or severe COVID-19 patients. Using Orbitrap MS-based targeted proteomics, Bezstarosti et al. detected tryptic peptides of SARS-CoV-2 proteins (NCAP, VME1) that could be utilized as a diagnostic of COVID-19.<sup>41</sup> Moreover, proteomics using LC–MS for COVID-19 identified 27 proteins as signatures with disease severity where 22 of them were upregulated, while the remaining 5 were downregulated with increasing severity of COVID-19.<sup>42</sup> Over 6739 proteins were identified using LC–MS-based global proteome analysis of PBMCs from COVID-19 patients, and specifically, this study identified that the nsp9 and nsp10 proteins from SARS-CoV-2 interact with host NKR, a NF- $\kappa$ B repressor, and may precipitate the strong interleukin (IL)-8/IL-6 mediated chemotaxis of neutrophils and increased host inflammatory response in COVID-19 patients.<sup>43</sup> Using nano-HPLC/nano-ESI-Orbitrap-MS/MS, Ihling et al. identified unique peptides originating from SARS-CoV-2 nucleoprotein from a highly diluted gargle solution of COVID-19 patients.<sup>44</sup> A cancer cell model system infected by SARS-CoV-2 followed by LC–MS-based proteomics revealed that diverse nucleic acid metabolism was critical for corona virus replication and suggested an inhibitor of nucleotide pool biosynthesis as a therapeutic for COVID-19 (Table 1).<sup>45</sup> Using MALDI-MS combined multivariate statistical analysis, Dattero et al. showed the potential of MALDI-MS as a complementary diagnostic tool for discriminating SARS-CoV-2 from other viruses.<sup>46</sup> Overall, LC and MALDI-MS based proteomics has shown potential as a key COVID-19 research area; however, development of rapid and sensitive MS techniques along with trials in larger clinical studies are necessary to translate the beneficial aspects of MS-based proteomics in pandemics like COVID-19 disease (Figure 1).

### ■ MASS SPECTROMETRY BASED GLYCOMICS IN COVID-19

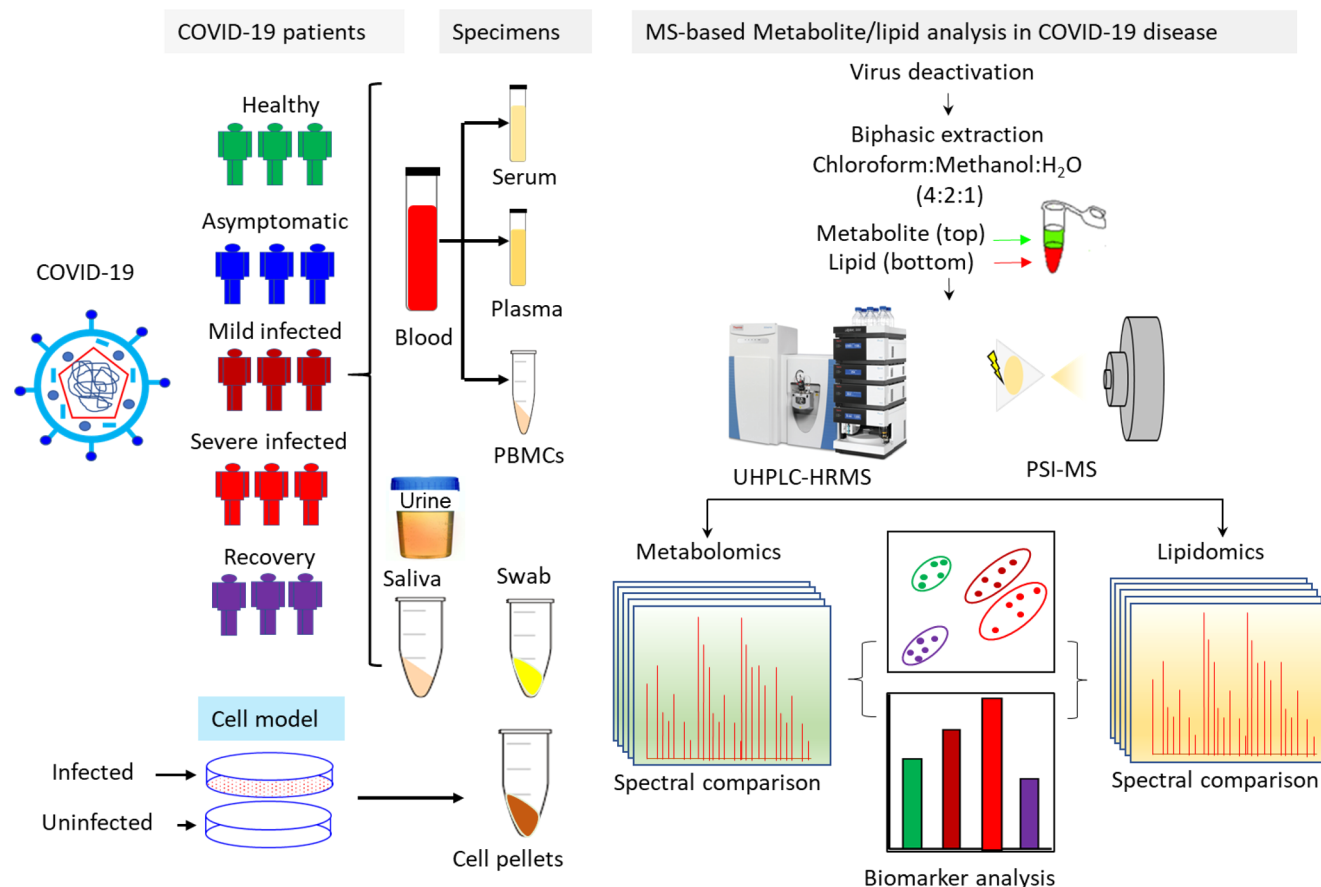
Glycomics analyzes the comprehensive study of glycomes, the entire complement of sugars, whether free or present in other molecules. Glycosylation is one of the most common forms of post-translational modifications of proteins, but glycan are also key players in lipid modification such as in galactosylcerebrosides and phosphatidylinositols. In many cases, bacteria and viruses utilize carbohydrate-carbohydrate interactions to infect the host, and this phenomena could be pertinent to the ongoing COVID-19 pandemic.<sup>47</sup> Extensive glycosylation of virus spike protein has been reported in MARS and SARS and offers a potential pathway to study for treatment.<sup>48</sup> Indeed, heavy glycan occupancy along with three novel O-glycosylation

sites was observed in ACE2 receptor infected by SARS-CoV-2.<sup>49</sup> In another study, using LC–MS, SARS-CoV-2 infection showed glycans mimic the polypeptide epitope that modulates Spike-ACE2 interactions.<sup>50</sup> Based on limited studies of glycans role in COVID-19, one can infer that glycans are one of the key players not only in the viral envelope but also on host receptor binding. Hence, understanding glycoscience in SARS-CoV-2 infection is another meaningful window to guide current vaccine development policy for COVID-19 pandemic.

### ■ MASS SPECTROMETRY BASED LIPIDOMICS IN COVID-19

MS-based lipidomics analyzes the targeted or global lipid profile (e.g., fatty acids, glycerophospholipids, sphingolipids, glycerolipids, glycolipids, and steroids) and its perturbation with disease or infection.<sup>51</sup> Lipidomics has had a tremendous impact in emerging pathogen studies. Lipids play important roles at various stages of host–pathogen interactions and pathogenesis.<sup>52</sup> For example, MS-based lipidomics revealed that arbovirus which is transmitted by *Aedes aegypti* mosquitoes leading to dengue, Zika, chikungunya, and yellow fever, utilizes increased levels of glycerophospholipids, sphingolipids, and fatty acids for viral replication.<sup>29,53</sup> Studies based on electron tomography identified that significant rearrangements of the host cell membrane architecture occur upon infection.<sup>54,55</sup> As the host cell membrane is enriched with lipid molecules, viral entry into the host requires direct or indirect association or modulations of host membrane lipids. Evidently, lipids play multifaceted roles in viral entry into the host cell.<sup>56</sup> Glycosphingolipids composed of a lipid and carbohydrate moiety have been shown as receptors for members of the nonenveloped viruses such as calicivirus (it includes genera of norovirus, sapovirus, vesivirus, lagovirus, neovirus, and recovirus), rotavirus, polyomavirus, and parovirus families.<sup>57</sup> The carbohydrate moiety of host cell membrane glycosphingolipids helps to direct the interaction between a host cell and the nonenveloped virus protein coat. This interaction leads to the virus internalization and initiation of infection.<sup>56–58</sup> Lipid receptors such as LDLR (low density lipoprotein receptor) act as an indirect receptor for hepatitis C virus (HCV) and bovine viral diarrheal virus (BVDV) where these viruses initially interact with LDL (low density lipoprotein) lipid in the bloodstream and then the virus is transported to the proximity of the target cell that will be infected via the association with the LDL–LDLR complex and eventually facilitates infection.<sup>59</sup> In the case of dengue, bis(monoacylglycerol)phosphate (BMP) and phosphatidylserine (PS) act as viral fusion cofactors for endosomal acidification-dependent fusion machinery.<sup>60</sup> After initial receptor interactions, several viruses such as influenza, HIV, and Ebola use lipid microdomains, known as membrane rafts and made by sphingolipids and cholesterol, as cofactors for entry into the host cell, assembly, and budding.<sup>56,61</sup>

It has been shown that viruses use lipids as signaling molecules for replication and infection. For example, phosphatidylinositol (PI) signaling influences HCV and enterovirus replication,<sup>62,63</sup> cholesterol biosynthesis stimulates Nile and dengue virus replication,<sup>64,65</sup> phosphatidylserine (PS) promotes Ebola virus replication,<sup>66–68</sup> and sphingomyelin (SM) and membrane lipid rafts are involved in Ebola, Marburg, and influenza virus infection,<sup>69–72</sup> while inhibiting *de novo* ceramide (Cer) synthesis significantly decreased ceramide accumulation and enhanced influenza A/H1N1 replication.<sup>73</sup>



**Figure 2.** MS-based metabolomics and lipidomics to study COVID-19. Recent metabolomics and lipidomics study on COVID-19 pandemic was used to develop this workflow. SARS-CoV-2 virus with different symptomatic patient conditions or infected cell models are shown. Typically, serum, plasma, and PBMCs from blood and other biomatrices such as urine, saliva, swab, and breath specimens can be utilized for targeted or untargeted metabolomics or lipidomics analysis in COVID-19 patients. MS spectral comparison followed by multivariate statistical analysis can be utilized to study metabolite or lipid-based biomarker with different level of COVID-19 severities. For detail about different COVID-19 metabolomic and lipidomic study sample preparation, data collection, and analysis, see Table 1.

LC–MS-based human blood plasma lipidome analysis identified that Ebola infection promotes profound modulation in global lipid profiles where PS, phosphatidylethanolamine (PE), Cer, phosphatidylglycerol (PG), and diacylglycerol (DG) were shown as increased with fatalities and suggested potential therapeutics toward targeting lipids such as PS in antiviral therapy.<sup>74</sup> Ultra-High performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry (UPLC-ESI-Q-TOF-MS)-based global lipidomics analysis of human Huh-7 cells uninfected or infected with MERS-CoV or HCoV-229E demonstrated that elevated levels of lysophosphatidylcholine (lysoPC), lysoPEs, and fatty acids (FAs) assisted with the replication of HCoV-229E and MERS-CoV viruses.<sup>75</sup> As lipids play numerous indispensable cellular functions and are involved in multiple steps in the virus life cycle, a global understanding of the role lipids play in COVID-19 infection could provide potentially significant insights about virus pathogenesis and management. As a frontline study, using LC-ESI-MS/MS, Wu et al. identified that DGs, FAs, and triglycerides (TGs) were identified in higher abundance in the fatality group as well as increased with the deterioration of the disease. In contrast, phosphatidylcholines (PC) were found as gradually reduced lipids over the course of COVID-19 fatalities. Overall, this study observed dyslipidemia

in COVID-19 patients and provides evidence of potential therapeutic strategies.<sup>76</sup>

In another study, using UPLC–MS/MS, Shen et al. reported increased levels of PCs and 21-hydroxypregnenolone, the essential intermediate for synthesizing corticosterone, but decreased levels of glycerophospholipid and notably down-regulated sphingolipids in both nonsevere and severe COVID-10 patients.<sup>39</sup>

As there is no specific vaccine/treatment available for COVID-19, knowledge from lipidome studies can be useful to develop druggable targets. For example, bioactive lipids such as oleoylethanolamide (OEA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are known from existing viral pathogenesis to inactivate enveloped viruses and inhibit microbial proliferation/replication.<sup>77–79</sup> As SARS-CoV-2, SARS, and MERS are all enveloped viruses, AA, EPA, and DHA can be applied to inactivate these virus infections and their appropriate doses may reduce morbidity and mortality due to COVID-19 and other similar conditions.<sup>77–79</sup>

Pandemic influenza as well as the emergence of novel respiratory viruses SARS-CoV, SARS-CoV-2, and MERS-CoV cause acute respiratory symptoms due to the production of sputum, a lipid rich component, at the respiratory tract.<sup>80,81</sup> It is evident from the discussion that bursts of lipid accumulation

are likely due to viral replication kinetics. In contrast, a study identified development of hypolipidemia in patients with COVID-19 symptoms where the degree of hypolipidemia positively correlated with the disease severity.<sup>82</sup> Overall, lipidomic studies have shown several critical insights about the role of diverse lipid molecules in COVID-19 pathogenesis. It will be a useful resource to correlate lipid profiles with therapy or vaccine outcomes as well as potential of drug targets to control the COVID-19 pandemic. Moreover, a larger study of COVID-19 patients with different clinical conditions should be subjected to global lipidome analysis to uncover the detailed role lipids play in COVID-19 patients (Figure 2).

## ■ MASS SPECTROMETRY BASED METABOLOMICS IN COVID-19

MS-based metabolomics studies targeted or untargeted metabolites (e.g., carbohydrates, amino acids, organic acids, amines, nucleotides, aliphatic, and aromatic compounds) and their perturbation with disease or infection.<sup>83,84</sup> Metabolites are transformed chemical entities during cellular metabolism and serve as direct signatures of biochemical activity; therefore, their levels are exquisitely sensitive to a wide range of perturbations linked to disease onset, infection, and/or pathogenesis.<sup>83,85,86</sup> MS-based untargeted metabolomics is performed as a global analysis of metabolite profiles in any given biological material and has the potential to uncover thousands of metabolites and link them to their cellular networks.<sup>83,87</sup> Recently, MS-based untargeted metabolomics has been utilized in discovery of metabolite-based biomarkers for the diagnostic of many diseases with unmet challenges.<sup>88–92</sup> MS-based metabolomics has been applied in several pandemic and epidemic diseases to identify potential therapeutic targets. For example, LC–HRMS-based metabolomics has been used to identify chemical ligands that can block nucleoprotein, a promising antiviral druggable target of Ebola and Marburg viruses.<sup>93</sup> Using UHPLC–Q-TOF MS based global metabolomics, Tian et al. observed that H1N1 influenza virus modulates energy metabolism including TCA cycle, lipid, nucleotide, and glutathione metabolism to efficiently carry out its life cycle in the host cell.<sup>94</sup>

In COVID-19 patients, using an LC-ESI-MS/MS-based targeted metabolomics approach, Wu et al. reported malic acid of the TCA cycle, carbamoyl phosphate of the urea cycle, and guanosine monophosphate (GMP) of nucleotide biosynthesis were profoundly altered in COVID-19 fatal patients (Table 1).<sup>95</sup> In a separate study, using UPLC–MS/MS-based untargeted metabolomics, Shen et al. performed molecular profiling of COVID-19 sera and identified significant activation of the kynurenine pathway with elevated kynurenate, kynurenine, and 8-methoxykynurenate in COVID-19 patients. In contrast, another study found decreased levels of choline and its derivative phosphocholine as well as global suppression of amino acids, and derivatives such as glutamate, arginine, *N*-(*I*-arginino)succinate, citrulline, ornithine, glutamine, 2-oxoglutarate, *N*-acetyl-L-glutamate, urea, fumarate, argininate, asymmetric dimethylarginine, symmetric dimethylarginine, homoarginine, and *N*-acetylarginine were observed in the sera of nonsevere COVID-19 patients compared to healthy (Table 1).<sup>39</sup> Similarly, using both UHPLC–MS-based targeted and untargeted metabolomics, Thomas et al. identified increased levels of kynurenine, glucose, and free fatty acids but widespread decreased levels of nitrogen metabolism including creatine, creatinine, polyamine, and circulating

amino acids in COVID-19 patients (Table 1). Viral replication and host cell homeostasis both depend on the four nicotinamide adenine dinucleotide (NAD) coenzymes, NAD<sup>+</sup>, NADH, NADP<sup>+</sup>, and NADPH, which are the central catalysts of metabolism.<sup>96,97</sup> Using LC–MS/MS-based targeted metabolomics, Heer et al. identified dysregulated NAD in SARS-CoV-2 infected cellular models.<sup>98</sup> As MS-based metabolomics techniques allow us to measure many metabolites relating to a wide variety of viral infection and pathogenesis, large-scale comprehensive studies on COVID-19 can assist scientific research to better understand this pandemic and potential treatments (Figure 2).

## ■ SAMPLE PREPARATION AND ITS CHALLENGES IN COVID-19 STUDY

Emerging pathogen sample types, sample processing, and development of their specific extraction methods for MS-based technologies are challenging but critically important. In COVID-19, saliva, nasopharyngeal swabs (NS), gargle solution, sputum, bronchoalveolar lavage fluid (BLF), and exhaled breath condensate (EBC) can be utilized for detection of SARS-CoV-2 particles. As SARS-CoV-2 spreads via respiratory droplets, both BLF and EBC could be more appropriate biospecimens for COVID-19 diagnosis.<sup>99–102</sup> In particular, EBC with or without COVID-19 patients could be promising for SARS-CoV-2 detection. Saliva or NS or gargle solution sampling is recommended for early diagnosis; in contrast, sputum or BLF sampling should be used for detecting and monitoring COVID-19 as the lower respiratory tract yields higher viral load with disease severity.<sup>102</sup> Urine, plasma, serum, and PBMCs from COVID-19 patients and diverse cellular models with viral infection or drug treatment can be used to interpret the molecular mechanism for diagnosis of COVID-19. During COVID-19 specimen handling, an MS lab should follow proper PPE rules, processing of inactivated virus or its containing specimens should be conducted in at least BSL-2 cabinet, and samples should only be stored in a proper designated freezer. Inactivation of SARS-CoV-2 or its containing materials is a major challenge in emerging pathogen sample preparation. However, different chemicals such as acetone, acetone/methanol, methanol/chloroform, UV at 254 nm, heat treatment of 65 °C or greater, alkaline (pH > 12) or acidic (pH < 3) conditions, or formalin can be used to inactivate coronavirus in controlled laboratories at biosafety level 3 (BSL3).<sup>103</sup> A multiomics sample preparation technique should be explored for integrated omics analysis of COVID-19 (Figure 2). Interestingly, MPLEx (metabolite, protein, and lipid extraction) was developed for emerging pathogen including SARS corona virus to partition a single sample into three distinct parts (metabolites, proteins, and lipids) while simultaneously inactivating the virus by solubilizing and disrupting the viral envelope and denaturing viral proteins.<sup>27</sup> Notably, COVID-19 MS coalition of MS laboratories has been established and working since the beginning of this pandemic to provide guidance and protocols on MS technologies-based sample collection, processing, extraction, data collection, and analysis to reduce the harm caused by the SARS-CoV-2 virus pandemic. As COVID-19 continues to grow, MS-based multiomics methods should be developed to study disease prognosis, assess biomarkers for diagnosis and treatment, understand the impact of infective particles in the environment, and evaluate the response of vaccine and therapeutics.



## ■ FUTURE PERSPECTIVES

As a resource for MS-based COVID-19 research, this review explored the current knowledge of SARS-CoV-2 infections and pathogenesis in context to genomics, proteomics, glycomics, lipidomics, and metabolomics of diverse COVID-19 patient materials and cellular model systems. The rapid, sensitive, and specific diagnosis of SARS-CoV-2 by fast and unambiguous testing is widely needed in response to the current outbreak.<sup>23</sup> LC-MS-based technologies have been predominantly employed since the beginning of the COVID-19 pandemic to identify SARS-CoV-2 using proteomic markers, to understand drug targets, to evaluate drug efficacy, or to elucidate the molecular mechanism upon SARS-CoV-2 pathogenesis and disease severity. As COVID-19 continues to progress and the complexity of SARS-CoV-2 pathogenesis and symptoms continues to grow, the strength and beneficial aspects of MS and its allied technologies should be utilized to better understand the COVID-19 pandemic. One of biggest challenge in current frontline PCR techniques for COVID-19 detection is its inability to detect mutation and unknown pathogens. PCR-based diagnostic testing is predominantly based on the identification of individual genetic targets. MS techniques such as PCR-ESI-MS (polymerase chain reaction coupled to electrospray ionization mass spectrometry) represents a novel multiplexed method that has recently emerged for broad range pathogen testing. PCR-ESI-MS technology is capable of identifying nearly all known pathogens directly from clinical specimens as well as identifying genetic evidence/amplicon of unknown pathogens with a broad genetic amplification approach.<sup>104</sup> Therefore, possibilities and advantages of PCR-ESI-MS should be explored in COVID-19 diagnostics.

Rapid pathogen identification is a key aspect in an infectious disease outbreak such as COVID-19 for appropriate preventative measures. However, current COVID-19 diagnostic techniques such as PCR or immunoassay is insufficient because of reagent shortages that can lead to a reduction in turnaround time for disease detection. Recent developments of direct MS such as segmented flow (SF)-MS and paper spray ionization (PSI)-MS have shown the potential for rapid, sensitive, highly reproducible, and comparable results to conventional LC-MS in their ability to identify biomarkers in the diagnosis of cancer and the discrimination of various bacterial strains, respectively.<sup>105,106</sup> Notably, PSI-MS has emerged as a rapid (>30 s) analytical technique to discriminate bacterial strains from raw specimens with minimal to no sample preparation requirements; however, the capability of PSI-MS in viral detection has not been studied. PSI-MS should be explored as a method to differentiate different viral strains based on potential strain specific molecular signatures.

In addition, MALDI-MS has been effective as a proteomics tool because of its relatively high tolerance of mixtures and biological contaminants.<sup>107,108</sup> MALDI-MS is a rapid analysis technique developed between the 1980s and early 1990s, winning one fourth of the Nobel Prize in 2002 for the analysis of proteins. Since its inception, it has been employed for the analysis of DNA, proteins, peptides, lipids, and small molecules.<sup>109–113</sup> MALDI has also been translated to clinical diagnostics for microorganism identification of infections and is now widely used across the United States and Europe in bacteria identification.<sup>114</sup> Therefore, the application of MALDI-MS into emerging pathogen research has the potential

to add value to current COVID-19 detection as well as utilize current equipment present in many clinical laboratories.

However, many MS instruments are either high cost to obtain or require a significant lab footprint to operate for many clinics. Hence, the development or translational of portable MS can be very important for clinical lab adoption as well as the potential for remote testing and screening. Indeed, miniaturized MS has been around for a decade as a fast and portable analytical tool in space research, security, and food science but it often faces the challenge of false expectations.<sup>115</sup> With improve performance and sensitivity, miniaturized MS can have many potential applications including in disease outbreaks such as COVID-19.

It is likely that many different technologies will contribute to develop strategies for treatment such as antiviral compounds, bioactive molecules, purified plasma, monoclonal antibodies (mAbs), viral molecule analogous compound, and vaccines to eventually prevent COVID-19. Besides MS technologies, nuclear magnetic resonance (NMR) spectroscopy is also often use as a rapid, quantitative, and highly reproducible approach in a variety of systems biology research and could be applicable in disease outbreaks.<sup>116–120</sup> However, MS-based technologies have incredible potential in this space. For example, mAbs has shown a wide range of therapeutic benefit in COVID-19.<sup>121</sup> However, higher quality characterization of intact mAbs is important. Top-down MS-based proteomics is a comprehensive technique used to decipher intact protein structure with significantly higher detection efficiency of protein level sequence and post translational modifications (PTMs). Therefore, top-down MS can be utilized for global elucidation of mAbs structure and PTMs by fragmentation.<sup>122</sup> Top-down MS can be applied in COVID-19 for identifying protein with immunogenic response as vaccine candidate.

Biomarkers associated with COVID-19 disease severity or treatment should be actively explored through an MS-based multiplex approach. Downregulated apolipoproteins and elevated nucleotide metabolism has been identified as a molecular signature with COVID-19 disease severity and suggest that these metabolic vulnerabilities can be further validated from a therapeutic standpoint. Consistent with several other pandemic and epidemic diseases, lipidomics of COVID-19 patients has shown association of lipid levels with disease severity. The role of FAs, glycerolipid, glycerophospholipid, and sterol lipids should be investigated with severe or mild COVID-19 patients. Bioactive lipids have been implicated as antiviral agents. Hence, therapeutic aspects of bioactive lipids such as OEA, AA, EPA, and DHA should be further explored for treating COVID-19. Accumulation of ceramide has been shown to negatively affect viral pathogenesis including influenza virus. Therefore, the detailed role of ceramide and its potential therapeutic benefit should be explored in COVID-19.

Similarly, in metabolomics, the kynurenine pathway has been implicated as a key elevated metabolic dependency as well as suppression of amino acid pools in COVID-19 patients. In addition, metabolomics has been utilized to predict antiviral drug efficacy in COVID-19.<sup>123</sup> Therefore, the level of metabolites should be actively explored with immune response, vaccine or drug efficacy. Diverse genetic or epigenetic factors have been identified with viral infection and pathogenesis and could be explored to better understand the host immune response and therapeutic target.<sup>124,125</sup> Different post-translational modifications such as glycosylation, ubiquitination,

acetylation, methylation, and phosphorylation have shown association with diverse pathogen infection, and their role should also be investigated in context with SARS-CoV-2 infection and disease progression in COVID-19.<sup>126,127</sup> Extracellular vehicles (EVs) such as exosomes have been identified as a molecular vehicle that can carry host and pathogen-derived nucleic acid, protein, metabolite, and lipid.<sup>128</sup> Hence, the role of EVs in SARS-CoV-2 viral infection, transmission, and pathogenesis should be explored in COVID-19.

Discovery of biomarkers and investigation of altered molecular networks through an integrated “omics” approach could be a valuable tool for better understanding of COVID-19. As proteomics, glycomics, lipidomics, and metabolomics are end products of upstream genomic and transcriptomics activities, future research should focus on integrated omics in COVID-19 which could help to better understand the molecular mechanisms of infection, pathogenesis, and therapeutic targets. As antiviral strategies are broadly based on directly targeting the virus or indirectly targeting the virus via host molecular modulation, MS-based multiplexing technologies could open critical knowledge in these fields for defeating the COVID-19 pandemic.

Overall, since the current testing capacity by conventional PCR based methods is insufficient due of shortages of global supplies such as RNA extraction kits and PCR reagents, alternative and/or complementary testing assays should be developed. Here, we suggest that targeted and untargeted MS-based multiplexing technologies could have potential to accelerate the understanding of the complexity of COVID-19 pandemic and its management.

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I.M. conceptualized and wrote the manuscript. T.G. reviewed and edited the manuscript before submission.

### Notes

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